CLAIMS

1. A liquid pharmaceutical composition in the form of an aqueous solution comprising an erythropoietin glycoprotein product having the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells, a multiple charged inorganic anion, a pharmaceutically acceptable buffer, said anion and said buffer being present in said solution in an amount to provide the solution with a pH of from 5.5 to about 7.0 and said product being present in said solution in a sufficient amount to provide a therapeutically effective amount of said product when the solution or a portion of said solution is administered to a patient.

2. The composition of claim 1 wherein said solution is an isotonic solution.

3. The composition of claim 1 wherein the anion is an anion of a multiple charged strong inorganic acid.

4. The composition of claim 3 wherein the anion is selected from the group consisting of sulfate, citrate or phosphate.

5. The composition of claim 4 wherein the anion is a sulfate anion.

6. The composition of claim 1 wherein the pH is 5.8 to 6.7

7. The composition of claim 6 wherein the pH is 6.0 to 6.5

8. The composition of claim 7 wherein the pH is about 6.2.

9. The composition of claim 1 wherein the buffer is selected form the group consisting of a phosphate or arginine/H_2SO_4/Na_2SO_4 buffers.

10. The composition of claim 9 wherein the buffer is a phosphate buffer in an amount of from 10 to 50 mmol per liter of said solution.

11. The composition of claim 1wherein the product is a human erythropoietin.

12. The composition of claim 11 wherein the product is expressed by endogenous gene activation.

13. The composition of claim 12 wherein the erythropoietin has the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:2.

14. The composition of claim 1 wherein the erythropoietin product has the sequence of human erythropoietin modified by the addition of from 1 to 6 glycosylation sites.

15. The composition of claim 14 wherein the sequence modification is selected from the group consisting of:
$Asn^{30}Thr^{32}$;
$Asn^{51}Thr^{53}$,
$Asn^{57}Thr^{59}$;
Asn^{69};
$Asn^{69}Thr^{71}$;
$Ser^{68}Asn^{69}Thr^{71}$;
$Val^{87}Asn^{88}Thr^{90}$;
$Ser^{87}Asn^{88}Thr^{90}$;
$Ser^{87}Asn^{88}Gly^{89}Thr^{90}$;
$Ser^{87}Asn^{88}Thr^{90}Thr^{92}$;
$Ser^{87}Asn^{88}Thr^{90}Ala^{162}$;
$Asn^{69}Thr^{71}Ser^{87}Asn^{88}Thr^{90}$;
$Asn^{30}Thr^{32}Val^{87}Asn^{88}Thr^{90}$;
$Asn^{89}Ile^{90}Thr^{91}$;
$Ser^{87}Asn^{89}Ile^{90}Thr^{91}$;
$Asn^{136}Thr^{138}$;
$Asn^{138}Thr^{140}$;
Thr^{125}; and

$Pro^{124}Thr^{125}$.

16. The composition of claim 1, wherein said glycoprotein product has the sequence of human erythropoietin modified by a rearrangement of at least one glycosylation site.

17. The composition of claim 16, wherein the rearrangement comprises deletion of any of the N-linked glycosylation sites in human erythropoietin sequence with the addition of an N-linked glycosylation site at position 88 of the sequence of human erythropoietin.

18. The composition of claim 17, wherein the sequence modification is selected from the group consisting of
Gln^{24} Ser^{87} Asn^{88} Thr^{90};
Gln^{38} Ser^{87} Asn^{88} Thr^{90}; and
Gln^{83} Ser^{87} Asn^{88} Thr^{90}.

19. The composition of claim 1, wherein the glycoprotein product is a pegylated erythropoietin.

20. The composition of claim 19, wherein the pegylated erythropoietin is a conjugate of an erythropoietin glycoprotein having at least one free amino group wherein said glycoprotein has the sequence of human erythropoietin or has said sequence modified by the addition of from 1 to 6 glycosylation sites or by rearrangement of at least one glycosylation site; said glycoprotein being covalently linked to $[poly(ethylene\ glycol)]_n$ groups by a linker of the formula $-CO-(CH_2)_x-(OCH_2CH_2)_m-OR$ with the -CO of each poly(ethylene glycol) group forming an amide bond with one of said amino groups; wherein R is lower alkyl; x is 2 or 3; m is from about 450 to about 900; n is from 1 to 3; and the values of n and m being such that the molecular weight of the conjugate minus the erythropoietin glycoprotein is from 20 kilodaltons to 100 kilodaltons.

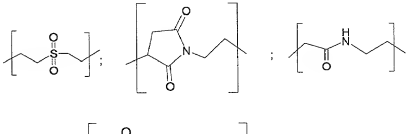
21. The composition of claim 20 wherein said pegylated erythropoietin protein product has the formula:

$$P\text{–}[NHCO\text{–}(CH_2)_x\text{–}(OCH_2CH_2)_m\text{–}OR]_n \qquad (I)$$

wherein m, n, x and R are as above, and P is the residue of the erythropoietin glycoprotein without the n amino group(s) which form the amide linkage(s) with the poly(ethylene glycol) group(s).

22. The composition of claim 36, wherein in formula (I) x is 2, m is 650 to 750, n is 1 and R is methyl.

23. The composition of claim 19, wherein the pegylated erythropoietin is a conjugate of an erythropoietin glycoprotein having at least one free amino group, said glycoprotein having the sequence of human erythropoietin or having said sequence modified by the addition of from 1 to 6 glycosylation sites; said glycoprotein covalently linked to from one to three lower-alkoxy poly(ethylene glycol) groups with each poly(ethylene glycol) group being covalently linked to the glycoprotein *via* a linker of the formula -C(O)-X-S-Y- with the C(O) of the linker forming an amide bond with one of said amino groups, X is $-(CH_2)_k-$ or $-CH_2(O-CH_2-CH_2)_k-$, k is from 1 to 10, Y is



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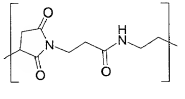

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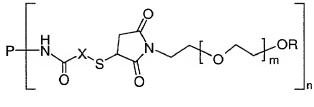
the average molecular weight of each poly(ethylene glycol) moiety being from about 20 kilodaltons to about 40 kilodaltons, and said conjugate having a molecular weight of from about 51 kilodaltons to about 175 kilodaltons.

24. The composition of claim 23 wherein said conjugate has the formula:



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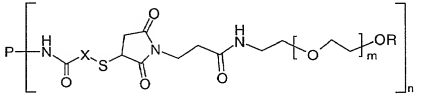
n is an integer from 1 to 3; m is an integer from 450 to 900; R is lower alkyl; X is $-(CH_2)_k-$ or $-CH_2(O-CH_2-CH_2)_k-$, and P is the residue of the erythropoietin glycoprotein without the amino group or groups which form an amide linkage; and k is as above.

25. The composition of claim 23 whewrein said conjugate has the formula:



wherein n is an integer from 1 to 3; m is an integer from 450 to 900; R is lower alkyl; X is $-(CH_2)_k-$ or $-CH_2(O-CH_2-CH_2)_k-$, and P is the residue of the erythropoietin glycoprotein without the amino group or groups which form an amide linkage; and k is as above.

26. A liquid pharmaceutical composition in the form of an aqueous solution comprising from 10 μg to 10,000 μg per ml of said solution of an erythropoietin glycoprotein product having the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells, from 10 to 200 mmol per liter of said solution of a multiple charged inorganic anion and from 10 to 50 mmol per liter of said solution of a pharmaceutically acceptable buffer, said anion and said buffer being present in said solution in an amount to provide the solution with a pH of from 5.5 to about 7.0.

27. The composition of claim 26 wherein said solution is an isotonic solution.

28. The composition of claim 26 wherein the anion is an anion of a multiple charged strong inorganic acid.

29. The composition of claim 28 wherein the anion is selected from the group consisting of sulfate, citrate or phosphate.

30. The composition of claim 29 wherein the anion is a sulfate anion.

31. The composition of claim 30 wherein the pH is 5.8 to 6.7

32. The composition of claim 30 wherein the pH is 6.0 to 6.5

33. The composition of claim 31 wherein the pH is about 6.2.

34. The composition of claim 26 wherein the buffer is selected form the group consisting of phosphate or arginine/H_2SO_4/Na_2SO_4 buffers.

35. The composition of claim 34 wherein the buffer is a phosphate buffer in an amount of from 10 to 50 mmol per liter of said solution.

36. The composition of claim 26 wherein the product is a human erythropoietin.

37. The composition of claim 36 wherein the product is expressed by endogenous gene activation.

38. The composition of claim 37 wherein the erythropoietin has the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:2.

39. The composition of claim 26 wherein the erythropoietin product has the sequence of human erythropoietin modified by the addition of from 1 to 6 glycosylation sites.

40. The composition of claim 39 wherein the sequence of modification is selected from the group consisting of:
$Asn^{30}Thr^{32}$;
$Asn^{51}Thr^{53}$,
$Asn^{57}Thr^{59}$;
Asn^{69};
$Asn^{69}Thr^{71}$;
$Ser^{68}Asn^{69}Thr^{71}$;
$Val^{87}Asn^{88}Thr^{90}$;
$Ser^{87}Asn^{88}Thr^{90}$;
$Ser^{87}Asn^{88}Gly^{89}Thr^{90}$;
$Ser^{87}Asn^{88}Thr^{90}Thr^{92}$;
$Ser^{87}Asn^{88}Thr^{90}Ala^{162}$;
$Asn^{69}Thr^{71}Ser^{87}Asn^{88}Thr^{90}$;
$Asn^{30}Thr^{32}Val^{87}Asn^{88}Thr^{90}$;
$Asn^{89}Ile^{90}Thr^{91}$;
$Ser^{87}Asn^{89}Ile^{90}Thr^{91}$;
$Asn^{136}Thr^{138}$;
$Asn^{138}Thr^{140}$;
Thr^{125}; and
$Pro^{124}Thr^{125}$.

41. The composition of claim 26 wherein said glycoprotein product has the sequence of human erythropoietin modified by a rearrangement of at least one glycosylation site.

42. The composition of claim 41, wherein the rearrangement comprises deletion of any of the N-linked glycosylation sites in human erythropoietin with the addition of an N-linked glycosylation site at position 88 of the sequence of human erythropoietin.

43. The composition of claim 42, wherein the sequence of a modification is selected from the group consisting of
Gln^{24} Ser^{87} Asn^{88} Thr^{90};
Gln^{38} Ser^{87} Asn^{88} Thr^{90}; and
Gln^{83} Ser^{87} Asn^{88} Thr^{90}.

44. The composition of claim 26, wherein said glycoprotein product is pegylated erythropoietin.

45. The composition of claim 44, wherein the pegylated erythropoietin is a conjugate of an erythropoietin glycoprotein having at least one free amino group wherein said glycoprotein has the sequence of human erythropoietin or has said sequence modified by the addition of from 1 to 6 glycosylation sites or by rearrangement of at least one glycosylation site; said glycoprotein being covalently linked to $[poly(ethylene\ glycol)]_n$ groups by a linker of the formula $–CO–(CH_2)_x–(OCH_2CH_2)_m–OR$ with the -CO of each poly(ethylene glycol) group forming an amide bond with one of said amino groups; wherein R is lower alkyl; x is 2 or 3; m is from about 450 to about 900; n is from 1 to 3; and the values of n and m being such that the molecular weight of the conjugate minus the erythropoietin glycoprotein is from 20 kilodaltons to 100 kilodaltons.

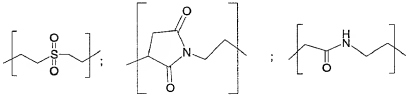
46. The composition of claim 45 wherein said pegylated erythropoietin protein product has the formula:

$$P–[NHCO–(CH_2)_x–(OCH_2CH_2)_m–OR]_n \qquad (I)$$

wherein m, n, x and R are as above, and P is the residue of the erythropoietin glycoprotein without the n amino group(s) which form the amide linkage(s) with the poly(ethylene glycol) group(s).

47. The composition of claim 46, wherein x is 2, m is 650 to 750, n is 1 and R is methyl.

48. The composition of claim 44, wherein the pegylated erythropoietin is a conjugate of an erythropoietin glycoprotein having at least one free amino group, said glycoprotein having the sequence of human erythropoietin or having said sequence modified by the addition of from 1 to 6 glycosylation sites; said glycoprotein covalently linked to from one to three lower-alkoxy poly(ethylene glycol) groups with each poly(ethylene glycol) group being covalently linked to the glycoprotein *via* a linker of the formula -C(O)-X-S-Y- with the C(O) of the linker forming an amide bond with one of said amino groups, X is $-(CH_2)_k-$ or $-CH_2(O-CH_2-CH_2)_k-$; and k is from 1 to 10; Y is



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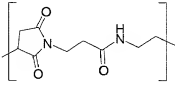
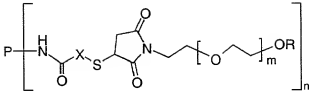


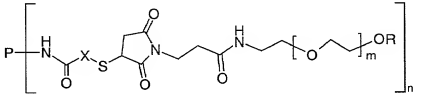
the average molecular weight of each poly(ethylene glycol) moiety being from about 20 kilodaltons to about 40 kilodaltons, and the molecular weight of the conjugate being from about 51 kilodaltons to about 175 kilodaltons.

49. The composition of claim 48 wherein said conjugate has the formula:



wherein n is an integer from 1 to 3; m is an integer from 450 to 900; R is lower alkyl; X is $-(CH_2)_k-$ or $-CH_2(O-CH_2-CH_2)_k-$, and P is the residue of the erythropoietin glycoprotein without the amino group or groups which form an amide linkage with X and k is as above above.

50. The composition of claim 49 whewrein said conjugate has the formula:



;

n is an integer from 1 to 3; m is an integer from 450 to 900; R is lower alkyl; X is $-(CH_2)_k-$ or $-CH_2(O-CH_2-CH_2)_k-$, and P is the residue of the erythropoietin glycoprotein without the amino group or groups which form an amide linkage; and k is as above.

51. The composition of claim 26 wherein said solution contains 10 μg to 10000 μg erythropoietin protein per ml of solution, from 10 to 200 mmol/liter of solution of the sulfate, and 10 to 50 mmol/liter of solution of phosphate, and a pH 6.0 to 6.5.

52. The composition of claim 51 comprising up to 20 mM per liter methionine, and 1 - 5 % of a polyol (w/v).

53. The composition of claim 52 comprising 10 μg to 10000 μg erythropoietin protein per ml of solution, 40 mmol/liter of solution of the sulfate, 10 mmol/liter of said solution of the phosphate, 3% mannitol (w/v), 10 mM methionine, and a pH 6.2.

54. The composition of claim 26 wherein said solution contains 10 μg to 10000 μg erythropoietin protein per ml of solution, 10 to 100 mmol/liter of solution of NaCl, 10 to 50 mmol/liter of solution of phosphate at a pH 6.0 to 7.0.

55. The composition of claim 54 wherein said solution comprises 10 μg to 10000 μg erythropoietin protein per ml of solution, 100 mmol/liter of solution of NaCl, 10 mM methionine, and 10 mmol/l phosphate, pH 7.0.

56. The composition of claim 26 wherein said solution comprises 10 μg to 10000 μg erythropoietin protein per ml of said solution, 10 to 50 mmol/liter of said solution of arginine, pH 6 to pH 6.5, 10 to 100 mmol/liter of solution of sodium sulfate.

57. The composition of claim 56 wherein said solution comprises 10 μg to 10000 μg erythropoietin protein per ml, 40 mmol/liter of solution arginine, pH 6.2, 30 mmol/l sodium sulfate, 3 % mannitol, 10 mM methionine, and 0.01% pluronic F68.

58. The composition of claim 26 wherein said solution comprises 25 to 2,500 μg/ml erythropoietin with the buffer anion and pH being

a) 10 mM sodium or potassium phosphate, 100 mM NaCl, and a pH 7.0 or
b) 10 mM sodium phosphate, 120 mM sodium sulfate, and a pH 6.2 or
c) 10 mM sodium phosphate, 40 mM sodium sulfate, 3% mannitol, and a pH 6.2 or
d) 10 mM sodium phosphate, 40 mM sodium sulfate, 3% mannitol, 10 mM methionine, and a pH 6.2 or
e) 40 mM arginine, 30 mM sodium sulfate, 3% mannitol, and a pH 6.2 or
f) 40 mM arginine, 30 mM sodium sulfate, 3% mannitol, 10 mM methionine, and a pH 6.2.

59. The composition of claim 26 wherein the amount of erythropoietin is 50, 100, 400, 800 or 2,500 μg/ml of solution.

60. The composition of claim 59 comprising 10 mM sodium phosphate, 40 mM sodium sulfate, 3% mannitol, 10 mM methionine, 0.01% pluronic F68, and a pH 6.2.

61. The composition of claim 60 comprising 40 mM arginine, 30 mM sodium sulfate, 3% mannitol, 10 mM methionine, 0.01% pluronic F68, and a pH 6.2.

62. A pharmaceutical composition in the form of lyophilisate or spray dried powder comprising an erythropoietin glycoprotein product having the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells, a multiple charged inorganic anion and a pharmaceutically acceptable buffer, said product anion and said buffer being present in said composition to provide upon addition to water a solution with a pH of from 5.5 to about 7.0 with said product being present in said solution in a sufficient amount to provide a therapeutically effective amount of said product when the solution or a portion of said solution is administered to a patient.

63. The composition of claim 62 wherein the anion is an anion of a multiple charged strong inorganic acid.

64. The composition of claim 63 wherein the anion is selected from the group consisting of sulfate, citrate or phosphate.

65. The composition of claim 64 wherein the anion is present in the composition in an amount to provide upon addition to water a solution containing said anion in an amount of 10 to 20 mmol/liter of said solution.

66. The composition of claim 65 wherein said product is present in the compostion in an amount to provide upon addition towater a solution containing said product in an amount of from 10 μg to 10,000 μg per ml. of said solution.